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Epidemiological Studies in Monitoring Reproductive Effects

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The possible hazards of occupational exposure on the reproductive health of workers is of great interest. In this review, epidemiological study designs suitable for reproductive studies and sources of outcome and exposure data are described with a few examples. Studies have been conducted on hormonal changes, semen quality, fertility, and various outcomes of pregnancy, e.g., spontaneous abortion, congenital malformations, perinatal mortality, birth weight, and health and development of the children. Also, sex distribution of children has been investigated as a possible risk indicator in some recent studies. In epidemiological studies, retrospective or prospective cohort design, case referent and nested case referent designs have been used. The validity of epidemiological studies depend on reliable data on the health effect and the exposure. The registers on congenital malformations and on births and other outcomes of pregnancy are useful sources of data for epidemiological studies. The coverage of the register and the accuracy of its contents should be known if the register is used for research. Personal interview of the worker is an important source of information, although recall problems may weaken the quality of the data. The reliability of the answers may be increased by using a few complementary questions on possible medical confirmation of the event. If both the interview and register data are available, the reliability of the information increases, the same concerns exposure information also. Examples of some conducted studies are given.

Introduction

Practical guidelines on epidemiological study designs and methods suitable for reproductive epidemiology have been described elsewhere (*1–4*). The general principles of epidemiological methods are not treated in detail here, but the reader is referred to relevant textbooks (*5*). The approach here takes into consideration such situations where no registers of reproductive outcome are available.

Reproductive Outcomes Studied

The outcomes of pregnancy (spontaneous abortion, chromosomal aberrations in aborted fetuses, preterm birth, stillbirth, sex ratio, twinning, etc.) and the condition of the child (birth weight, neonatal death, genetic disease, congenital malformation, functional disturbances, cognitive development, childhood cancer) with respect to occupational exposure have been studied. Some reports describe the adverse effects of contaminated breast milk on breast-fed babies. There is also information on the changes of fertility and adverse effects of exposure to gonads or to the hormonal regulation of the reproductive cycle in men and women. Reviews and summaries have been published (*6–11*).

Recently, much interest has been focused on detecting very early, clinically unrecognizable abortions by following the excretion of human chorionic gonadotropin from women trying to become pregnant (*9*). Such a study, although it would give accurate information about the beginning of the pregnancy, is expensive, laborious, and time consuming and requires adequate laboratory facilities. It may also be difficult to find a sufficiently large group of women from some industrial branch willing to participate in such a study. Because the process of reproduction is complex, examining only one outcome may fail to reveal toxic effects. Therefore, it would be recommendable to examine several outcomes whenever possible.

Data Sources for Reproductive Outcome

The sources of the data on reproductive outcome depend on the outcome of interest. Personal interviews and postal questionnaires have been used to obtain information on pregnancy outcome. The reliability of questionnaire data may be weakened because of a low response rate and uncertainty in the recognition and recall of an abortion or a malformation. To elucidate this problem, the validity of questionnaire data on spontaneous abortions was investigated in a Swedish study (*12*). The questionnaire data were compared with the hospital records and hospital discharge register. Ninety-seven percent of the diagnoses received from the women who had answered affirmatively to two complementary questions on spontaneous abortion could be found in the records. The questions concerned a positive pregnancy test and whether a doctor had confirmed the

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miscarriage. The conclusion was that a questionnaire report of spontaneous abortion can be accepted without verification from medical records if the woman has answered these complementary questions affirmatively.

Socioeconomic status and educational level may influence the ability and the willingness to participate in interview and questionnaire studies. Furthermore, selection between respondents and nonrespondents by exposure or pregnancy outcome may cause bias. For example, exposed women who have experienced a spontaneous abortion may be more eager to participate in postal questionnaire studies than nonexposed women (*13*). Therefore, analysis of the pregnancies among nonrespondents is advisable.

Medical records or nationwide registers often are more reliable than questionnaire data because the information is based on medical diagnoses and recorded at the time of the event. A follow-up on the coverage and accuracy of the register should be conducted, however. The recorded data are not very sensitive, as far as the early abortions (especially the clinically unrecognizable spontaneous abortions) are concerned. Also, different care-seeking behavior may weaken the validity of the data. The educational level, economic factors, and availability of medical services may influence the use of the services. If only economically well-off women can use antenatal care services or deliver in hospitals, the women in the medical records would be selected according to their economic status, which might cause bias in the study. In such a situation, an alternative source for identifying the study population might be needed.

Example of a National Database on Pregnancy Outcome

In Finland, the Hospital Discharge Register (HDR), maintained by the National Board of Health of Finland, contains information on recorded hospitalized spontaneous abortions, induced abortions, and deliveries. The structure, data content, reliability, and coverage of HDR have been described recently (*14*). The computerized register covers 94% of all births and 85% of all induced abortions recorded officially by the Statistical Office of Finland. The coverage of spontaneous abortion data in the register has, according to two questionnaire studies, been from 80 to 83 % (*14*). Annually, 10–17% of the spontaneous abortions in Finland were treated polyclinically (as outpatients) in the years 1973–1983 (*14*). It is estimated that about 10% of the recognized spontaneous abortions are not treated in hospitals or polyclinics in Finland.

Registers of Congenital Malformations

Registries of congenital malformations exist in a number of European countries. Registers are based, for example, on specific notification of malformations (England and Wales, Finland, Sweden, Czechoslovakia, and Hungary) or birth notifications (Norway, Belgium, Sweden). EUROCAT is a network of specialized regional registries in Europe with central coordination (*15*).

According EUROCAT a registry for congenital malformations should meet the following criteria (*15*): *a*) the birth notification system must provide a population nominator and some demographic information (maternal age, geographic distribution of residence); *b*) the quality of diagnoses should be high using standard definitions and terminology; *c*) multiple sources of information should be used and linkage between the sources must be possible; *d*) case identification should be as little as possible biased by survival characteristics (stillbirths, neonatal deaths, induced abortions following prenatal diagnosis, and live births should be equally reported); and *e*) the analysis, validation, and follow-up of the collected data should be foreseen. For example, the Finnish Register of Congenital Malformations (FRCM), established in 1963, is based on compulsory notification of all malformations detected during the first year of life. The malformations of stillborn babies weighing over 600 g during 1963–1985 (over 500 g after the year 1985) are registered from compulsory death certificates and completed from the pathologist's autopsy records. The notification lists the names and dates of birth of the mother and child, and the type of malformation of the child. From 1986 onward, information on the type of work of the parents at the beginning of the pregnancy has been registered.

The structure of the register and surveillance studies based on FRCM have been reviewed (*16*). When registered data have been compared with the hospital information, a reporting failure of about 15–30% has been found. To this, the failure rate in detection should be added; according to the hospital comparisons, it is of the order of 30%. Hence, the underreporting in the FRCM has been estimated to be 45–60%. The underreporting of small anomalies is greater than that of severe malformations.

Birth Registers

In many countries there are also birth registers collecting information on the length of the gestation, the possible problems during the pregnancy, the vital status of the child at birth, weight and length of the child, and events during the neonatal period. In Finland, the Medical Birth Registry was introduced in 1987 with the purpose to provide data for planning, follow-up, evaluation, and development of health care and for epidemiological research. The registration is based on reports sent within 7 days after birth by the maternity hospitals to the National Agency for Wellfare and Health. The registry also collects information about the mothers' health and smoking habits during pregnancy. The coverage and validity of the registry has been found to be good regarding most variables (*17*).

Exposure Data

The information on occupational exposure can be collected from workers, employers, occupational health personnel, or from medical documents, when available. If more than one source of exposure data is available, the reliability of the exposure information is likely to be better. Also, occupational hygienic measurements and results of biolog-

ical monitoring may be used. The time of measurements in respect to the time of pregnancy or spermatogenesis has to be taken into account when the relevance of the measurement data on the exposure during the time of interest in respect to the studied reproductive outcome is considered.

Study Types

Cross-Sectional Studies

Descriptive cross-sectional studies give information on how common various adverse health effects, e.g., reproductive outcomes, are in the general population or in an occupational group. In a cross-sectional study, the exposure and the health effect are measured at the same time. Data may be collected from women in the general population or in certain occupations, from occupational health care centers, centers providing prenatal care, hospital records, etc. The frequencies of abnormal outcomes from normal pregnancies or births are calculated. The validity of the results depends on the coverage of the data source and the accuracy of the information obtained.

A cross-sectional design is seldom recommended for etiological studies on reproduction. In spontaneous abortion studies, a cross-sectional design may even lead to false results because women commonly leave their job during pregnancy or after having a child, and those having a spontaneous abortion return to work quickly. If those with a child do not return, the workplace might become "enriched" with women who experienced a miscarriage. To avoid such error, former workers should be included in longitudinal studies (*18*).

Cross-sectional studies and case reports may generate hypotheses on potential reproductive hazards, but the hypotheses at least in the pregnancy-related studies need to be tested by longitudinal studies. Cross-sectional design may may be feasible for studying fertility, semen quality, or hormonal levels (*1*).

Etiological Studies

In etiological studies, the potential associations between exposure and adverse reproductive outcome can be investigated. Cohort and case-referent studies have been conducted to reveal reproductive toxicants in the general environment and the work environment. Longitudinal designs, i.e., where the exposure and events are examined over a longer period of time, may be either prospective or retrospective. In a prospective study, the population is followed from a certain point forward and the information on the outcome and exposure collected. In a prospective study, the follow-up starts from the present time forwards. A prospective design would be ideal for a reproductive study because the exposure information could be obtained before the outcome of the pregnancy is known. Prospective studies are very laborious, time consuming, and expensive to conduct; therefore, retrospective studies are often seen to be more feasible.

Cohort Studies

The starting point for a cohort study is the exposure of interest. The studied group, the cohort, consists of people exposed to a suspected toxicant. The frequency of various adverse reproductive outcomes in the cohort is compared to that of an unexposed reference group or general population. In a cohort study only one exposure, but several outcomes, can be studied. To avoid confounding due to, for example, social class differences, the members of a referent group should be similar to the cohort group in all but one respect: they should not have the studied exposure.

In cohort studies, the risk estimate is the risk ratio (RR). That is the ratio of adverse outcome (e.g., of the pregnancy) in the exposed group divided by the ratio of the adverse outcome in the unexposed reference group:

$$\mathrm{RR} = \frac{a/(a+b)}{c/(c+d)}$$

where a is the number of adverse pregnancy outcomes in the exposed cohort; $a+b$ is the number of all pregnancies in the cohort; c is the number of adverse pregnancy outcomes in the reference group, and $c+d$ is the number of all pregnancies in the reference group.

Examples from Cohort Studies. In Finland, the screening of the occupational reproductive hazards began with register-based studies among female members of various labor unions. In the union of chemical workers, women in the pharmaceutical industry had an increased risk of spontaneous abortion for pregnancies occurring during the union membership or after membership (*19*). No individual data on exposure were available in this study.

Later, a case-referent study among workers, nested in the cohort of the workers of pharmaceutical factories, was conducted to test the hypothesis. In the latter study, exposure to organic solvents in general and to methylene chloride and estrogens increased the relative risk for spontaneous abortion significantly (*20*).

The Finnish Population Register was linked with the Hospital Discharge register in a study (*21*). The linkage was conducted by using the social security numbers. The relative risk of spontaneous abortion was analyzed by occupation. The relative risk of spontaneous abortion was significantly increased among female laboratory workers, laundry workers, weavers, butchers and sausage makers, and among women having contact with fur-bearing animals. The significant findings formed hypotheses for further case-referent studies, where individual exposure data has been collected.

Another possibility to study the relationship between the pregnancy outcome and effects of occupational exposure has been used in Montreal, Canada. There, 56,000 women were interviewed in 1982–1984 after delivery or spontaneous abortion in 11 obsterical units in Montreal. From 60 occupational groups, women in sales, services, and manufacturing sectors had evidence of adverse outcome of pregnancy. Spontaneous abortions were observed significantly in excess in nursing aides, in sales occupations and food and beverage service; stillbirth in agricul-

ture, horticulture, and leatherwork; congenital defects in women in child care, certain service occupations, and the manufacture of metal and electrical goods (*22*).

A prospective study has been conducted in a county of Sweden, where during 3 years, 3900 working women were interviewed by a questionnaire during their first visit at the prenatal care center (*23*). Data on occupational factors, social circumstances, and lifestyle factors were collected by in the questionnaires. There were no significant differences in the incidence of adverse pregnancy outcome (spontaneous abortion, perinatal death, birth defects, or low birth weight) between the nine occupational categories used.

A relatively new method to study slight decreases in fertility is to measure the time to pregnancy, i.e., how many menstrual cycles (with no contraception) it takes a couple to achieve a clinical pregnancy (*10*). For this information no registers are needed; the number of cycles can be obtained from the women at antenatal clinics, in the hospital of the delivery, or even afterwards in a personal interview or by a mailed questionnaire. The length of the time to pregnancy should be a sensitive indicator of a reproductive toxicant causing fertility loss. Baird et al. (*10*) estimate that to detect a 50% drop in mean fecundity (the probability of pregnancy in each cycle) would require data from 55 exposed and 55 unexposed women who are pregnant. So far, only a few such reports have been published. In one study the pharmacists handling antibiotics had an increased time to pregnancy compared to other personnel in pharmacies (*11*).

Case-Referent Studies

In case-referent studies the starting point is the disease or adverse health effect in question. The persons with an adverse pregnancy outcome are defined as cases, and referents are selected from among those who do not have an adverse outcome. The exposure of the cases is compared to the exposure of the referents. In a case-referent study, only one outcome at a time, but several exposures, can be studied.

In case-referent studies, the relative risk is expressed as an odds ratio (OR). It is calculated as the ratio of exposed and unexposed cases divided by the ratio of the exposed and unexposed referents:

$$\mathrm{OR} = a/b{:}c/d = ad{:}bc$$

where a is the exposed cases, b is the unexposed cases, c is the exposed referents, d is the unexposed referents. Logistic regression models can be used in the analyses of the case-referent studies (*24*). The relative risk, expressed as an odds ratio in the case-referent studies, is an indirect estimation of the risk of adverse pregnancy outcome among the exposed women in relation to the nonexposed ones.

Case-Referent Study Nested in a Cohort. The study may become more effective, i.e., the exposure density is enriched, if the cases and referents can be chosen within a cohort (e.g., in some broad occupational group, some industry, or some union of the workers). Such a study is called a nested case-referent study. The prerequisite for a suitable study base is that there are workers with different exposure levels (high, moderate, none). The study is effective only if there is a sufficient number of exposed cases for the analysis (*5*).

Examples. In Finland, a number of (nested) case-referent studies have been conducted to test the hypotheses based on the findings on register-based cohort studies (*25,26*). For example, a study concerning female workers in laundries and dry cleaners was started because of the suggestive finding in the cohort study among the members of the union of chemical workers (*19*), and in the study based on the Population Register (*21*). In the case-referent study, individual exposure data were collected. Exposure to terachloroethylene and work as a dry cleaner during the first trimester of pregnancy were associated with an increased risk of spontaneous abortion (*27*). A similar study in Sweden, however, did not show any significant risk of adverse pregnancy outcome (*28*).

In Finland, biological monitoring records have been used as an entry to a study base and as a source of information on individual exposure. As biological measurements are usually done on heavy and well-documented exposures, the monitored workers were considered particularly suitable for reproductive studies.

The effects of exposure to organic solvents and lead were studied among biologically monitored workers. In a case-referent study among women who had earlier been monitored biologically for organic solvent exposure, the odds ratios were significantly increased for exposure to aliphatic hydrocarbons, especially in graphic work, and toluene, especially in shoe work (*29*). Also, the pregnancy outcome of the spouses of men who had been monitored biologically for solvent exposure has been studied. Paternal exposure to organic solvents during spermatogenesis (80 days before the conception) was associated with an increased risk of spontaneous abortion in that pregnancy. The associations were significant for high exposure to toluene and to solvent mixtures of thinner type (*30*).

Control of Confounding

A confounder is a risk of the disease of interest which in a given study is associated with the exposure of interest. The woman's age at pregnancy, previous adverse pregnancy outcome, and lifestyle factors such as smoking, alcohol consumption, and use of drugs are considered as potential confounders.

Matching can be used, in part, to control for confounding. Matching the cases and referents for multiple factors will diminish the availability of suitable referents and thus decrease the number of case-referent combinations in the study. Matching by many factors may also lead to overmatching, i.e., if the matching has been carried too far, the cases and referents will be very similar even with respect to the exposure studied (*24*). In studies on pregnancy outcome, it may be sufficient to match the cases and referents only for age.

Logistic regression models are feasible for analyzing materials where several confounders have to be controlled for. The confounders are included in the analysis as variables. Logistic regression modeling yields an estimate of the risk, which is corrected for the estimated effects of potential confounders.

Conclusions

There are many uncertainties in the epidemiological studies on potential occupational reproductive hazards. The methodology has been developed to increase the reliability and validity of the studies. The credibility of the results increases if more than one well-conducted study shows results of the same direction and if the results are biologically plausible in the light of toxicological and experimental data.

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